Experimental Parasitology 139 (2014) 6–11

Contents lists available at ScienceDirect

Experimental Parasitology

journal homepage: www.elsevier.com/locate/yexpr

Toxoplasma gondii: Prevalence in species and genotypes of British bats (*Pipistrellus pipistrellus* and *P. pygmaeus*)

Nicole S. Dodd [a], Jennifer S. Lord [a,b], Robert Jehle [a], Steven Parker [b], Fiona Parker [b], Darren R. Brooks [c], Geoff Hide [a,c,*]

[a] Ecosystems and Environment Research Centre, School of Environment and Life Sciences, University of Salford, Salford M5 4WT, UK
[b] South Lancashire Bat Group, P.O. Box 512, Bury, Lancashire BL8 9FB, UK
[c] Biomedical Research Centre, School of Environment and Life Sciences, University of Salford, Salford M5 4WT, UK

HIGHLIGHTS

- Two UK bat species, *Pipistrellus* spp, were infected with *Toxoplasma gondii* (10.39%).
- *P. pipistrellus* prevalence: 9.9% (±7.0%; $n = 71$); *P. pygmaeus*: 16.67% (±29.8%; $n = 6$).
- Microsatellite genotyping revealed one major interbreeding group in *P. pipistrellus*.
- No significant association was observed between *T. gondii* infection and genotype.

GRAPHICAL ABSTRACT

Bat Species	*Toxoplasma* prevalence
Pipistrellus pipistrellus	9.9% ±7.0%
Pipistrellus pygmaeus	16.67% ±29.8%
Both species	10.39% ± 6.06%

ARTICLE INFO

Article history:
Received 1 October 2013
Received in revised form 7 February 2014
Accepted 9 February 2014
Available online 19 February 2014

Keywords:
Pipistrellus pipistrellus
Pipistrellus pygmaeus
Toxoplasma gondii
Genotyping
STRUCTURE
Bats

ABSTRACT

Few studies have investigated *Toxoplasma gondii* infections in bat populations and none have reported its presence in protected British bat species. Using a collection of dead/euthanased bats collected from Lancashire, UK, two species of bats (*Pipistrellus pipistrellus* and *Pipistrellus pygmaeus*) were tested using a highly sensitive SAG1-PCR method specific for detection of *T. gondii* DNA ($n = 77$; 71 *P. pipistrellus* and 6 *P. pygmaeus*). Whilst some potential bias may exist in the sampling strategy, an overall prevalence of 10.39% (±6.06%; 95%CI) was detected. All *P. pipistrellus*, were also genotyped using eleven polymorphic microsatellite loci to determine their local population structure. The programme STRUCTURE revealed that the majority of individuals (83%) were derived from one interbreeding population, and the remaining individuals (17%) had mixed genetic origins. There was no significant difference in the frequency of *T. gondii* infection or geographical distribution between subclusters. As all British bats are insectivorous, the routes of infection with *T. gondii* remain elusive. However, the locally large and panmictic gene pool suggests that intraspecies transmission could be applicable.

1. Introduction

Toxoplasma gondii is a globally distributed, obligate intracellular parasite with a complex lifecycle. It is of significant medical and economic importance as it causes abortion and congenital disease in humans and domestic animals. Three routes of transmission are thought to occur: ingestion of tissue cysts by intermediate hosts consuming raw infected meat, ingestion of oocysts shed by the

* Corresponding author at: Ecosystems and Environment Research Centre, School of Environment and Life Sciences, University of Salford, Salford M5 4WT, UK. Fax: +44 161 295 5015.
E-mail address: g.hide@salford.ac.uk (G. Hide).

http://dx.doi.org/10.1016/j.exppara.2014.02.007
0014-4894/

feline definitive host into the environment and the transplacental transmission of tachyzoites from mother to foetus (Dubey, 2009). Due to these multiple potential transmission routes, and global distribution, it is highly prevalent and is thought to be able to infect almost any warm-blooded animals if they have the opportunity to encounter an infective stage (Dubey, 2009). This is largely due to the ability of the parasite to propagate both sexually, through the cat as the definitive host, and asexually (Dubey, 2009; Hide et al., 2009).

Bats are one of the most abundant, widely distributed and diverse vertebrate groups, and exhibit a variety of behaviours, including living in close proximity, which may make them highly suitable for parasite transmission and dispersion (Nicholls and Racey, 2006). They carry a wide range of pathogens including viruses, fungi, bacteria, protozoa and helminths (Gardner and Molyneux, 1988; Mayen, 2003; Nathwani et al., 2003; Lord et al., 2012). Bats are recognised as important vectors of zoonoses (Nathwani et al., 2003) and as potentially rich sources of novel emerging viruses (Calisher et al., 2006). The recent emergence of the fungal pathogen, White Nose Syndrome, has also been of considerable concern for bat conservationists (Blehert, 2012).

Despite concerted effort in other areas of bat parasitology, driven by emerging infectious diseases in humans, livestock and bat populations themselves, very few studies on *T. gondii* infection in bats have been reported. In addition, despite the close contact between individual bats, there is no obvious mechanism of initial infection that easily complies with the accepted transmission routes (Hide et al., 2009). Using serological techniques, Zetun et al. (2009) failed to detect *T. gondii* in vampire bats (*Desmodus rotundus*) in Brazil, whilst Smith and Frenkel (1995) did not detect *T. gondii* in the big brown bat (*Eptesicus fuscus*) in Kansas, USA. *Toxoplasma* was, however, isolated from the tissues of two bat species from Kazakhstan, (Galuzo et al., 1965). In two separate surveys of zoo animals, a serologically positive Indian flying fox was reported in Seoul, Korea, (Choi et al., 1987) and two clinical cases of toxoplasmosis were reported in black flying foxes in Australia (Sangster et al., 2012). Cabral et al. (2013) were the first to isolate and genotype *T. gondii* from bats using bioassays in mice. They found two infected individuals from a collection of 369 bats. Using a PCR detection system, a recent study of 550 insectivorous bats, collected in southern Myanmar, showed a prevalence of 29.3% infected with *T. gondii* (Sun et al., 2013). In another recent study from China (Yuan et al., 2013), *T. gondii* was detected by serology in 18.4% of a sample of 217 bats representing five species. To our knowledge no other studies, as yet, have reported *Toxoplasma* in bats, and no studies have so far been conducted in Europe (but see Dodd et al., 2010, based on preliminary results from a limited subset of animals used in the current study).

The discovery of high prevalences of *T. gondii* in insectivorous bat species (Dodd et al., 2010; Sun et al., 2013; Yuan et al., 2013) raises interesting questions about parasite acquisition. Transmission could occur during contact within roosts, by incidental infection with oocysts from the environment or perhaps by vertical transmission from mother to offspring. These questions could be addressed by investigating the distribution and genetic relationships of *T. gondii* infected bats in a sympatric population. Some recent studies have investigated bat population structure in relation to zoonotic disease infection (Turmelle and Olival, 2009; Atterby et al., 2010; Smith et al., 2011). None, however, have investigated the population structure of bats in relation to *T. gondii* infection.

The objectives of our study were to investigate the prevalence of *T. gondii* in a population of pipistrelle bats in the UK, to investigate host population structure and to investigate the prevalence of infection in relation to any substructuring observed within the bat population.

2. Methods

2.1. Bat sample collection

A collection of two species of British bats *Pipistrellus pipistrellus* ($n = 71$) and *Pipistrellus pygmaeus* ($n = 6$) was provided by The South Lancashire Bat Group (Registered Charity No. 1109519). These animals were collected over a two year period across the sampling area (South Lancashire, UK). The *P. pipistrellus* ($n = 71$) were collected throughout the Lancashire area with the majority of individuals found between Bolton and Burnley and a few within Manchester city centre. *P. pygmaeus* ($n = 6$) were all collected at the same location (Walkden). In UK law, bats are protected species making it extremely difficult to catch and kill bats without very good reason. For this reason, all bats were found dead, later died during rehabilitation or were euthanased by a veterinary surgeon. (We recognise that this approach may have an impact on potential collection bias and the sample size). All procedures were conducted in accordance with UK law and had no impact on conservation (Lord et al., 2012). The heads from the bats were tested by the Veterinary Laboratories Agency (VLA, Weybridge) to screen for the presence of European bat lyssavirus as required by law in the UK. No bats were found to be positive. UK Health and Safety law, required us to keep bat tissues frozen, in a secure location until absence of lyssavirus infection was confirmed, thus preventing from the isolation of parasites for genotyping. The location where the bats were collected was georeferenced to allow distribution mapping. Within the *P. pipistrellus* collection, a total of 28 (39.4%) females and 43 (60.6%) males were represented revealing a non-significant male bias ($\chi^2 = 2.024$, df = 1, $P = 0.1548$). The study received ethical approval from the University of Salford Research Governance and Ethics Committee (RGEC Reference: REP09/095).

2.2. DNA extraction, PCR detection of T. gondii and DNA sequencing

Due to the large proportion of the brain removed for lyssavirus testing, this study used sections of CNS tissue that were approximately 2 mm^3 in size. For DNA extraction, samples were placed in 400 µl lysis buffer, incubated overnight in Proteinase K and extracted with Tris buffered phenol:chloroform:isoamyl alcohol 25:24:1 pH 8.0 (VWR International Ltd, UK) as previously described (Thomasson et al., 2011). Extracted DNA was visualised using agarose gel electrophoresis to confirm presence, quality and amount. Quality of extracted DNA samples, for PCR analysis, was ensured by amplification of the mammalian alpha-tubulin gene (F: 5′-CGTGAGTGCATCTCCATCCA-3′ and R: 5′-GCCCTCACCCACATACCAGTG-3′) as previously described (Morley et al., 2005, 2008). The detection of *T. gondii* was based on a nested PCR method targeting the surface antigen 1 gene, (Thomasson et al., 2011). Extensive precautions were taken to avoid PCR contamination as described previously (Williams et al., 2005; Hughes et al., 2008). Bands obtained by nested PCR were sequenced to ensure that they were the correct 519 bp product. PCR amplification products were purified prior to being sequenced (Source Bioscience (UK)) using a Geneflow (UK) Q-Spin gel extraction purification kit. The data was compared to published *T. gondii* sequences by multiple sequence alignment using the CLUSTAL W software alongside a reference SAG1 sequence taken from the Genbank database (Accession number: GQ253086.1). Also the BLAST algorithm search (http://www.ncbi.nlm.nih.gov/BLAST/) was used to confirm that sequences derived from PCR amplified bands were SAG1 products.